



# 3D Normal Human Neural Progenitor Tissue-Like Assemblies: A Model of Persistent Varicella Zoster Virus Infection and a Platform to Study Viral Infectivity and Oxidative Stress and Damage

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# Varicella-Zoster-Virus

- Alpha herpesvirus
- One of 8 human herpesviruses
- Causes Chicken Pox (Varicella) and **Shingles (Zoster)**
- Live attenuated vaccine
- Licensed in the US 1995
- >120 million doses (1995-2011, USA)
- Vaccine prevents chickenpox
- Establishes persistent infection (latency)
- Latent virus will reactivate







# VZV in astronauts

before  
0 / 42

	VZV copies / ml saliva		
flight days	subject 1	subject 2	subject 3
AME			
-133	0	0	0
-131	0	0	0
-129	0	0	0
-127	0	0	0
-125	0	0	0
-123	0	0	0
-121	0	0	0
-119	0	0	0
-125	0	0	0
-117	0	0	0
-115	0	0	0
-113	0	0	0
-111	0	0	0
-109	0	0	0
-10			



during  
11 / 36

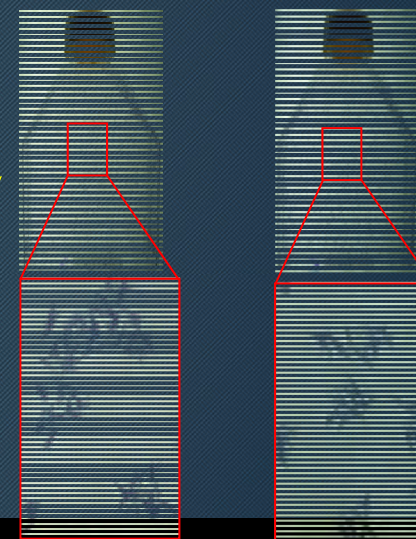
2	224	18	0
3	0	247	0
4	0	0	0
5	128	0	0
6	0	0	0
7	200	0	0
8	0	0	0
9	2500	650	0
10	0	75	0
11	450	0	0
12	0	0	0
13	120	23	0

105 total samples  
after  
7 / 27

14			
15	1250	560	0
16	45	0	0
17	0	340	0
18	110	45	0
19	0	23	0
20	0	0	0
21	0	0	0
22	0	0	0
23	0	0	0

subject 1

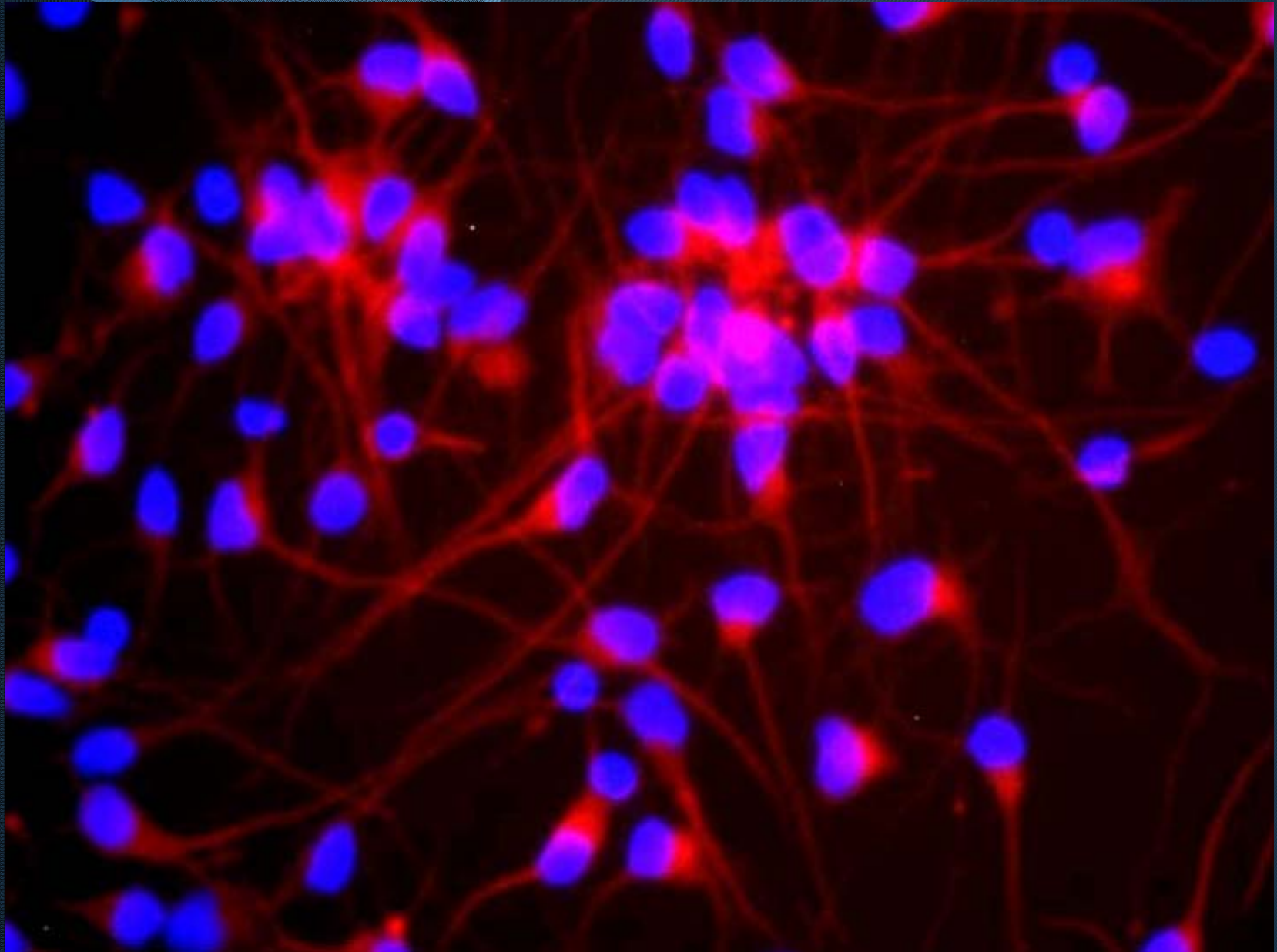
subject 2



$\alpha$ VZV immunostain



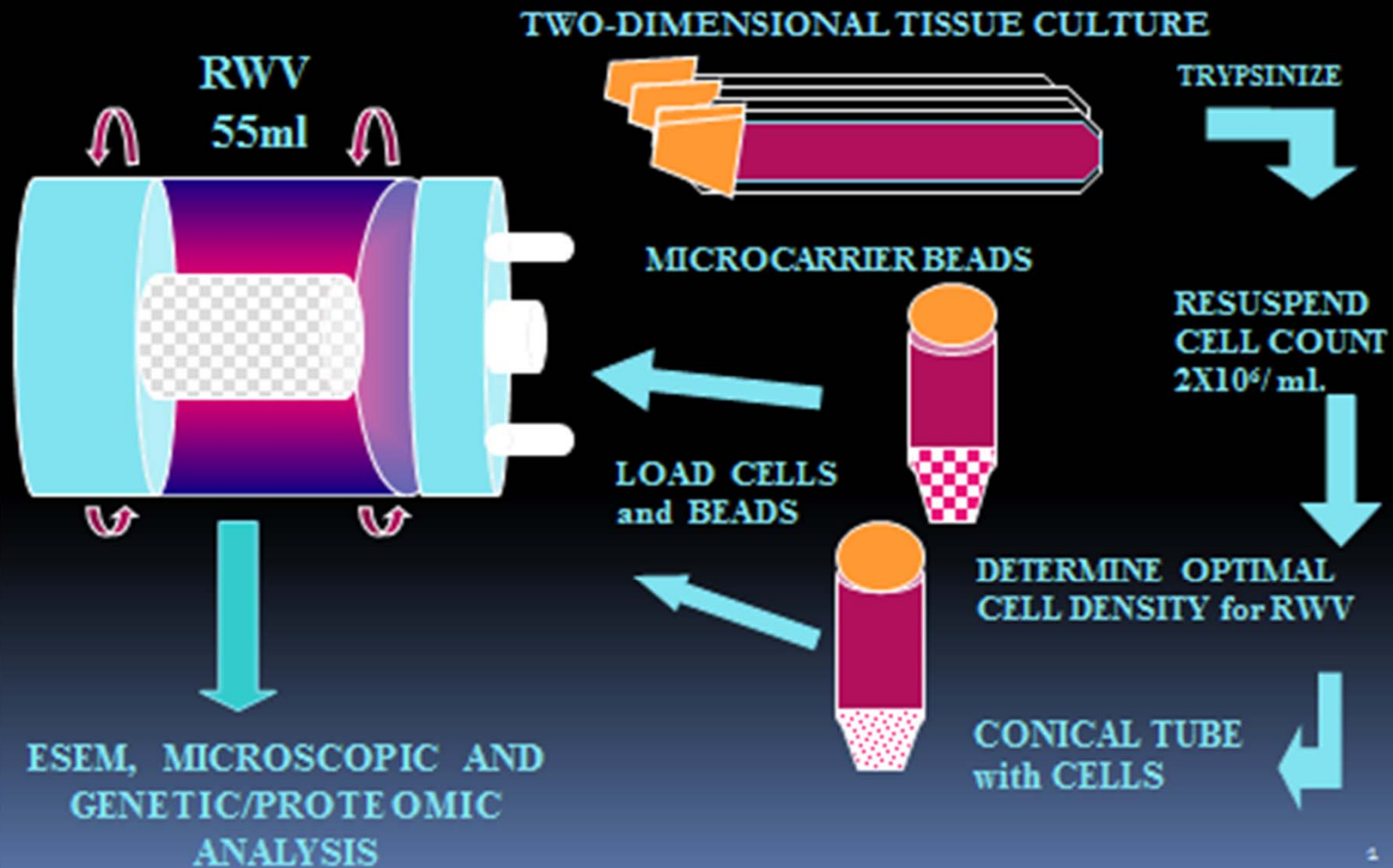
## 2D Normal human Neural Progenitor (NHNP) Cells







## CONSTRUCTION OF THREE - DIMENSIONAL TISSUE ASSEMBLY INTO ROTATING WALL VESSEL (RWV)



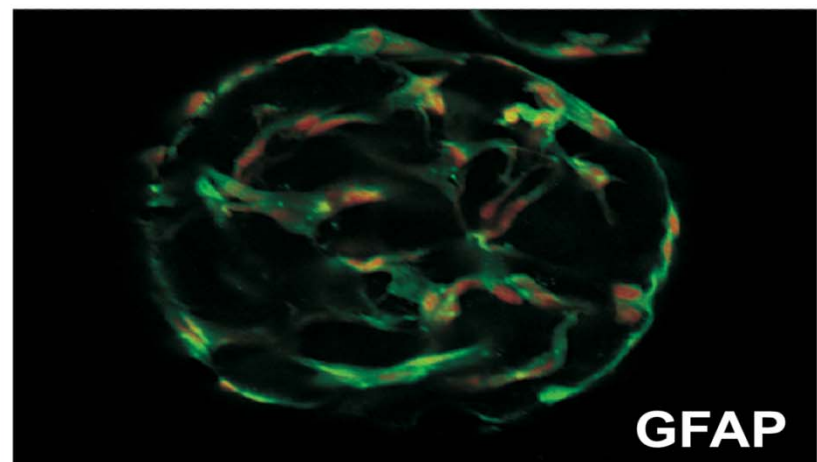
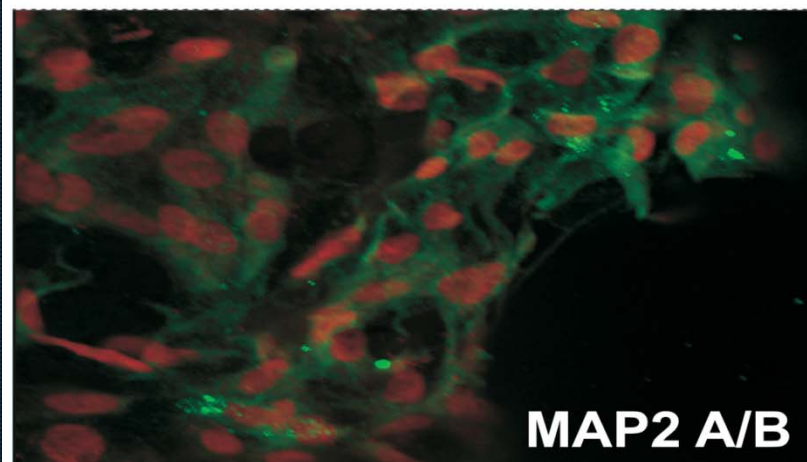
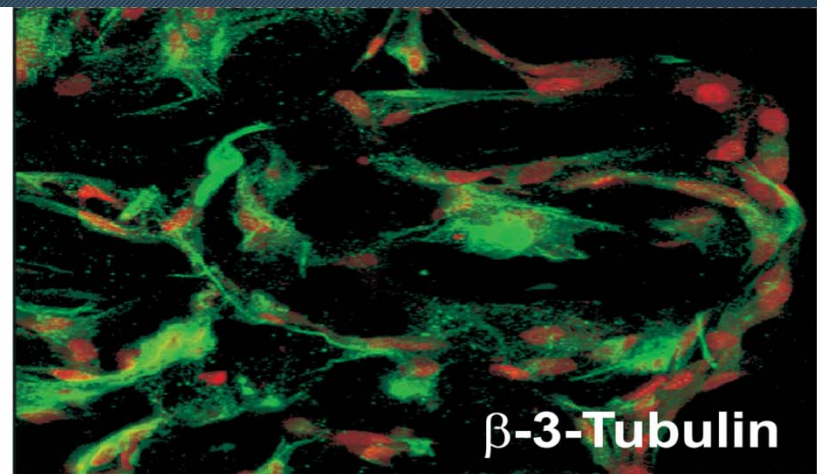
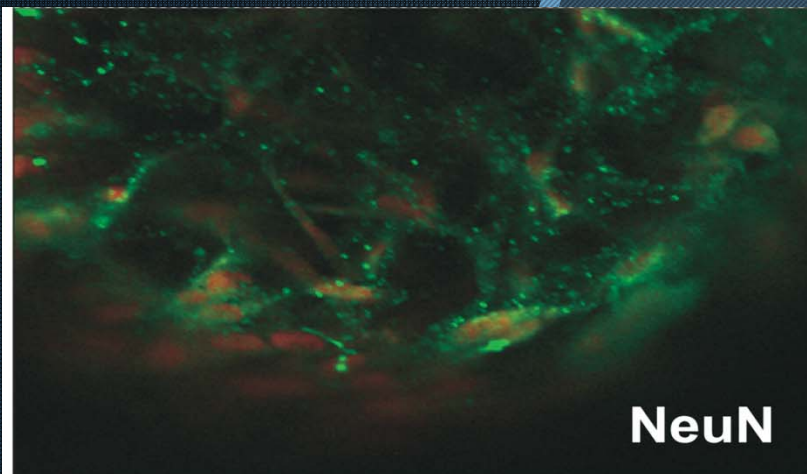


# Characteristics of TLA

Can be cultured for >6 months

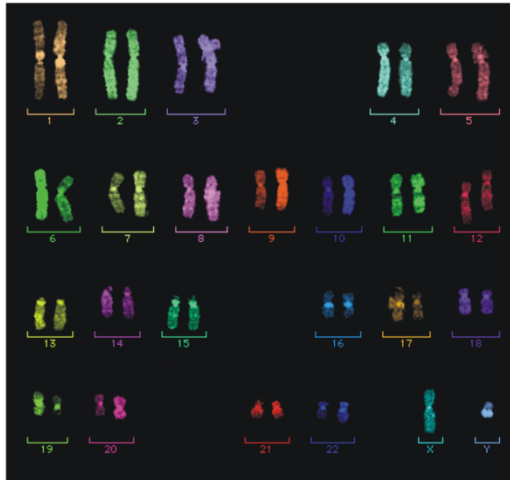
Similar expression profile as primary Trigeminal ganglia

Genetically stable (FISH)

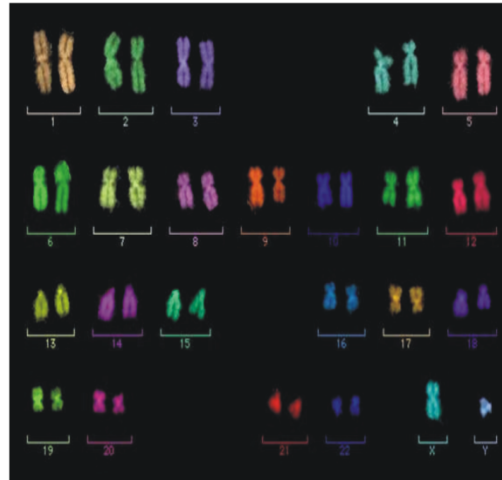




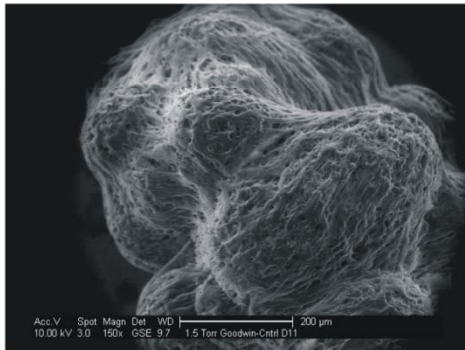
A.



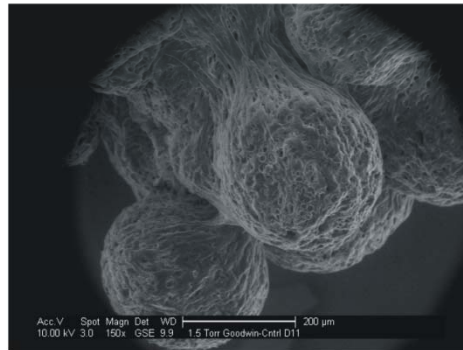
B.



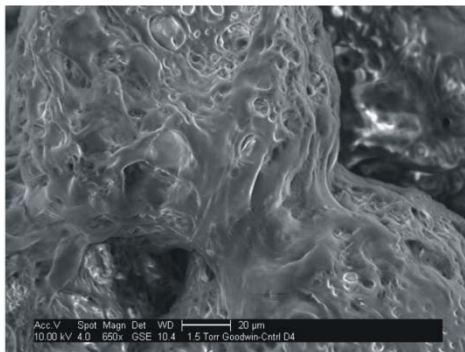
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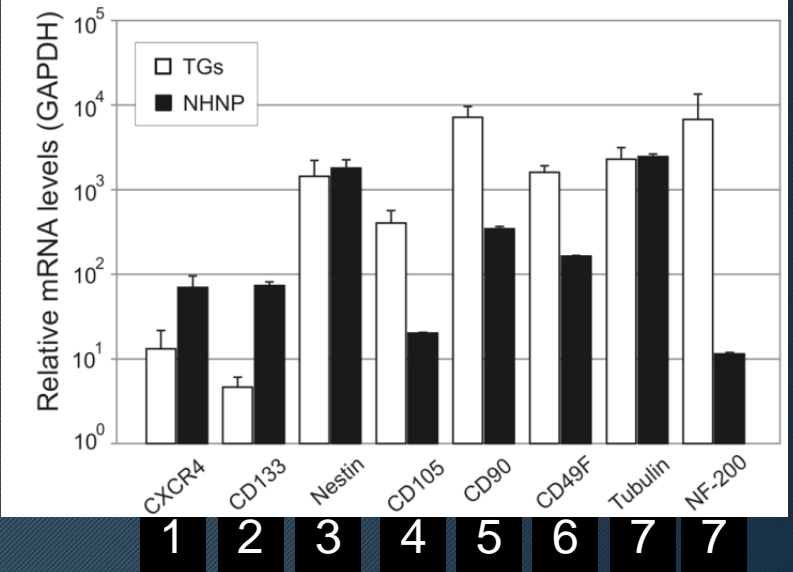
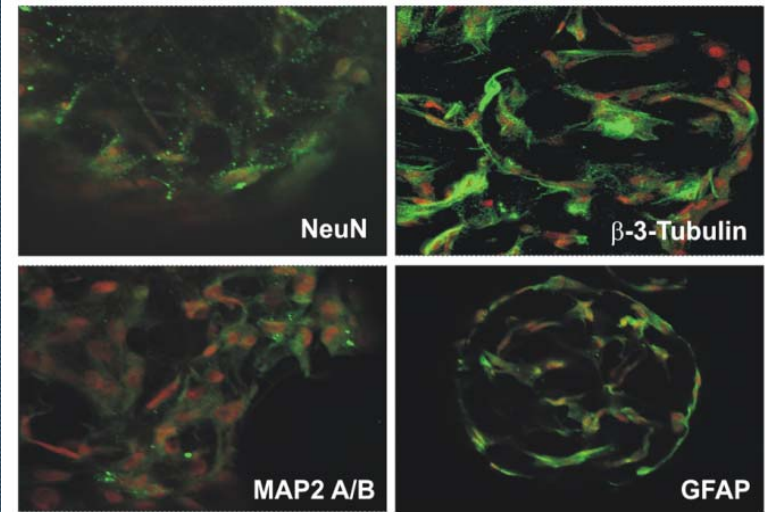
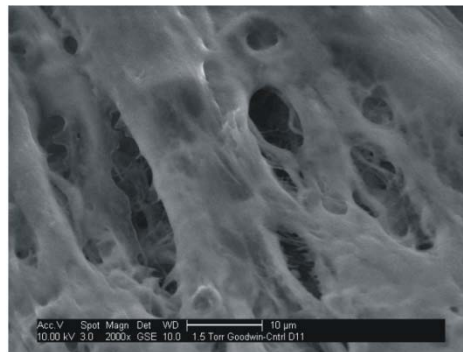
D.



E.

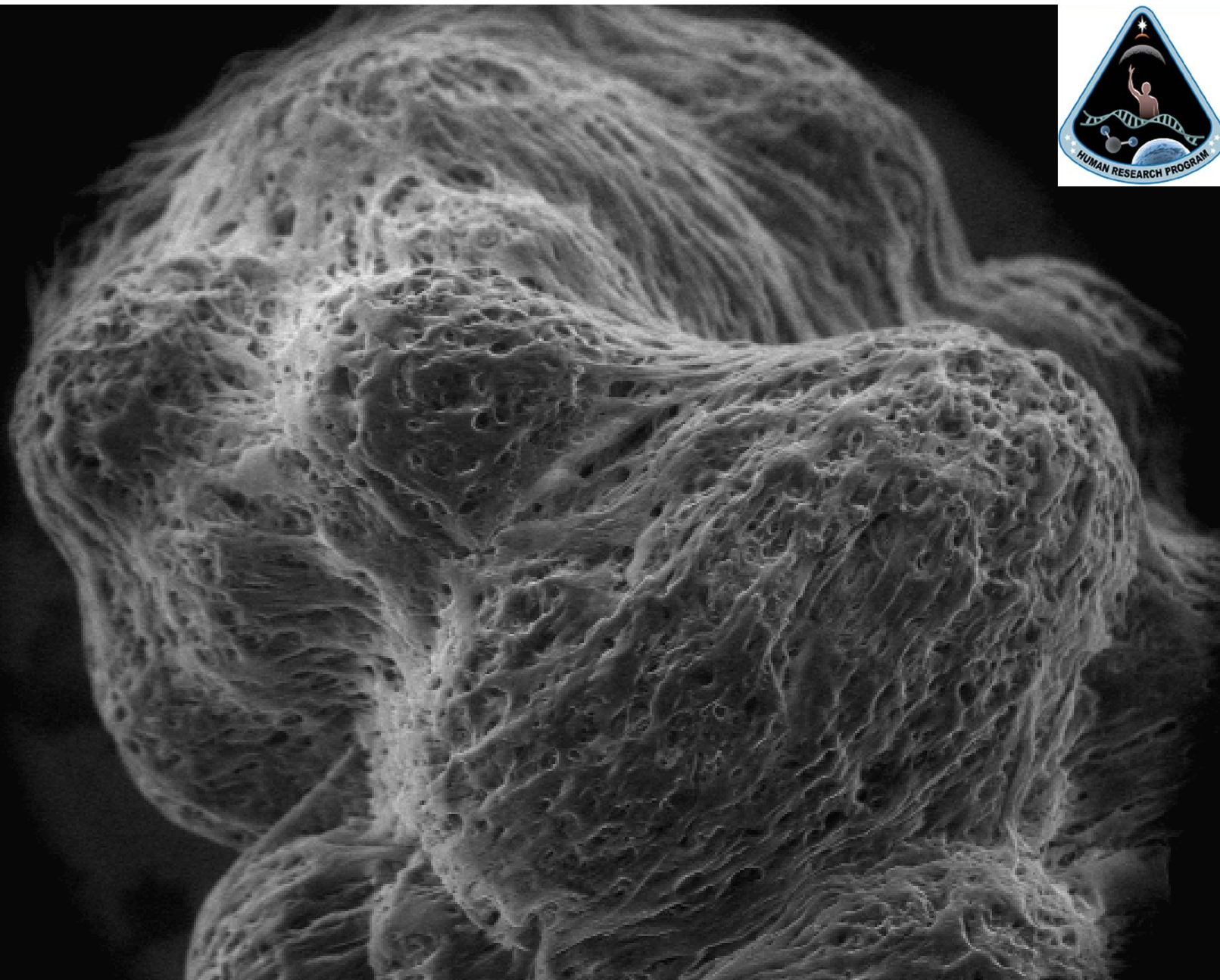


F.



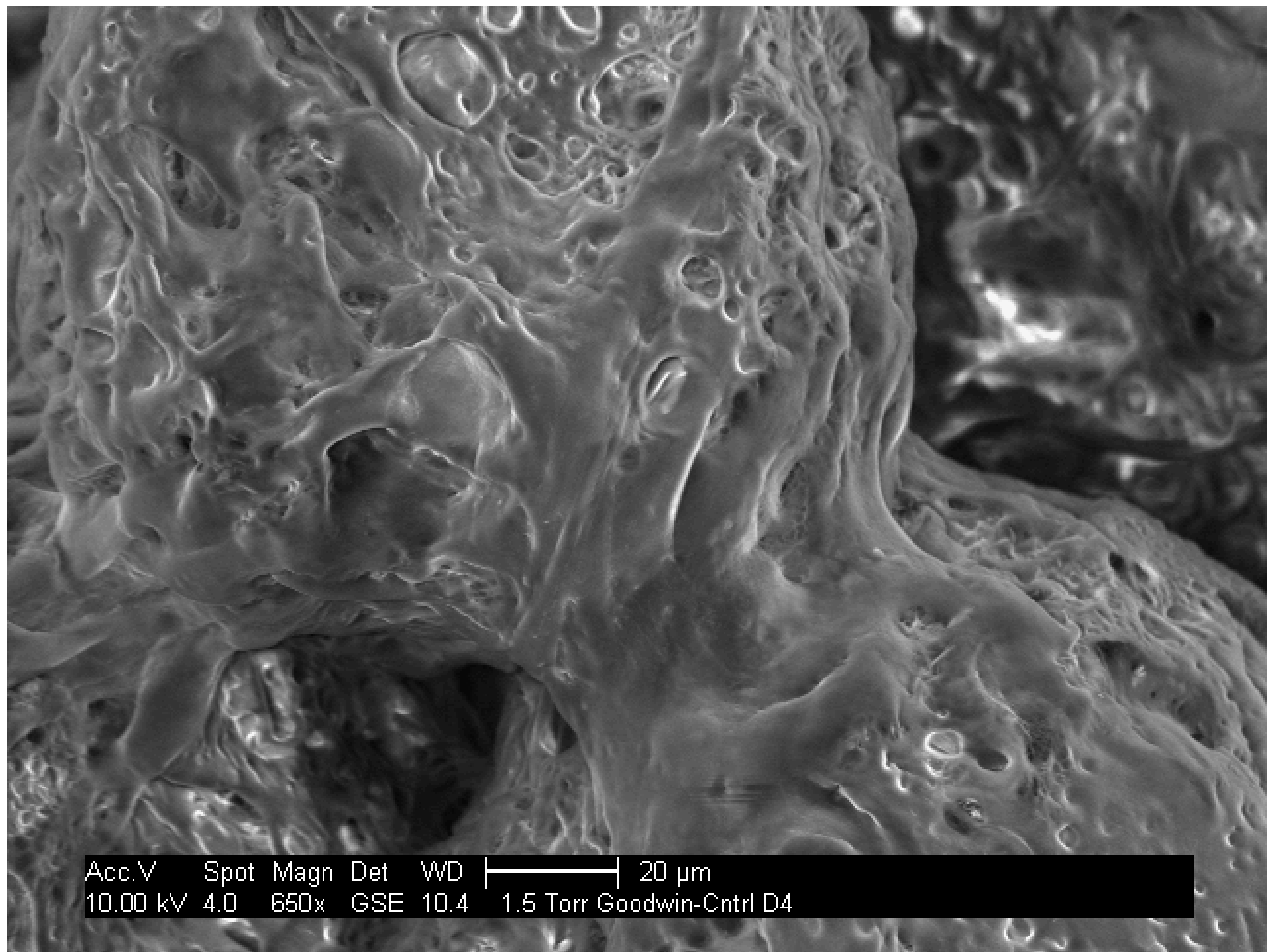
1. neural progenitor
2. primitive neural stem cell
3. neuronal precursor
4. neural stem cell
5. neuronal progenitor
6. early progenitor maturation
7. fully differentiated neuron





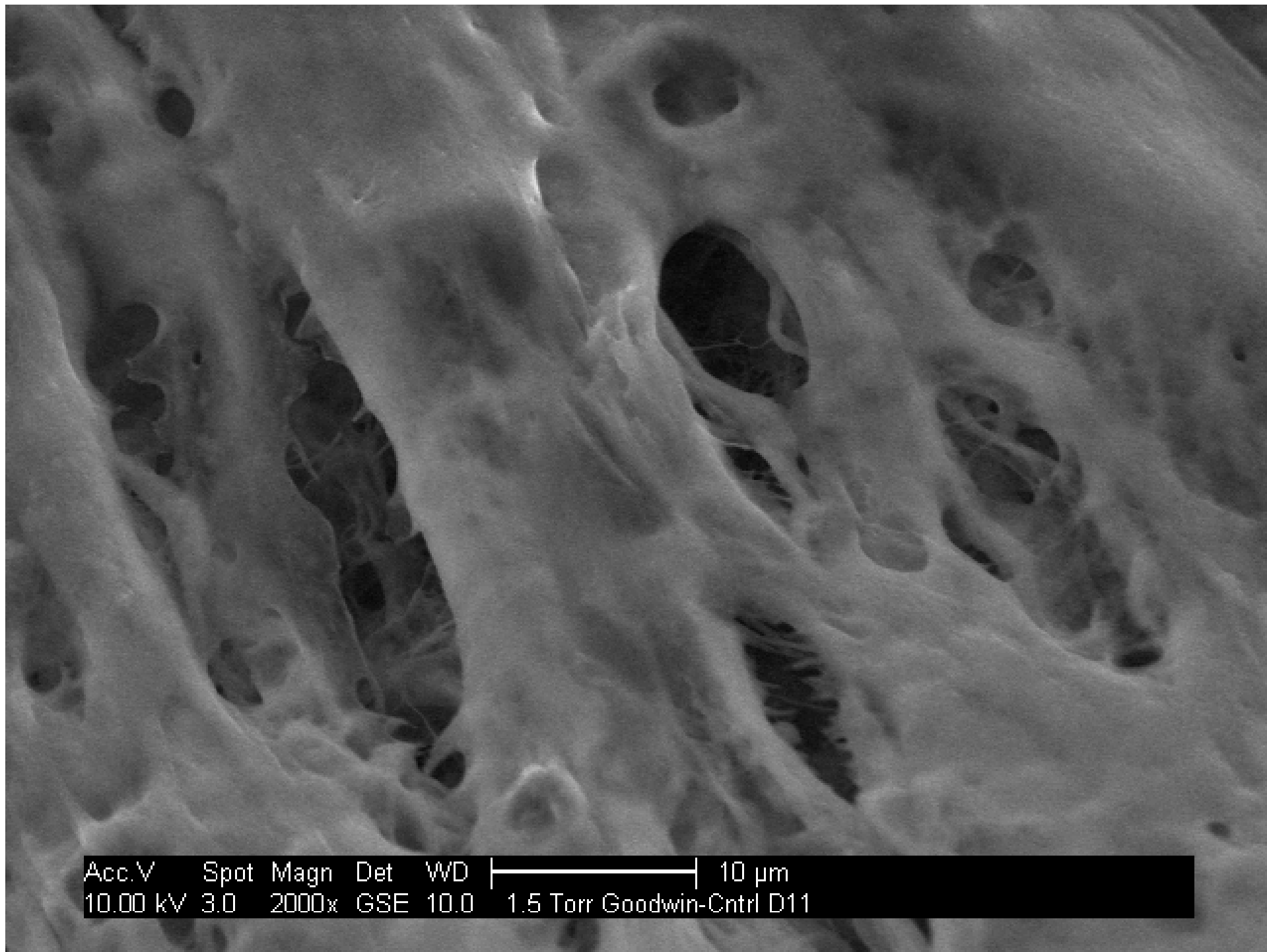
Acc.V	Spot	Magn	Det	WD		200 $\mu$ m
10.00 kV	3.0	150x	GSE	9.7	1.5 Torr Goodwin-Cntrl D11	





Acc.V Spot Magn Det VWD | 20 μm  
10.00 kV 4.0 650x GSE 10.4 1.5 Torr Goodwin-Cntrl D4

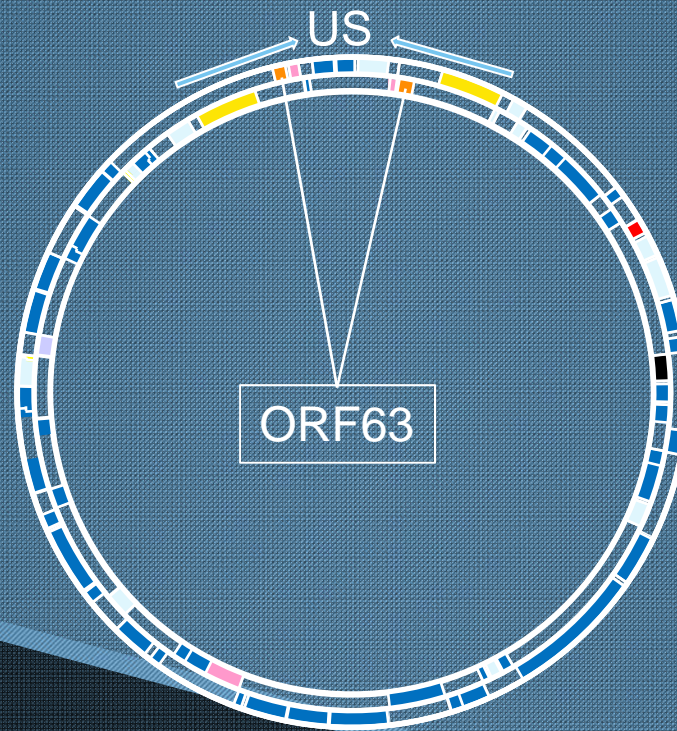
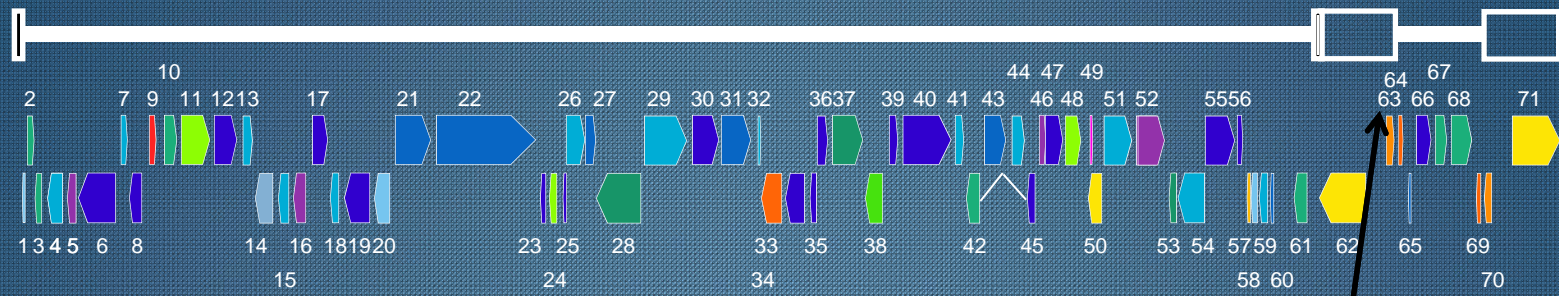




Acc.V	Spot	Magn	Det	WD		10 µm
10.00 kV	3.0	2000x	GSE	10.0	1.5 Torr Goodwin-Cntrl D11	



# Varicella Zoster (Chicken Pox)



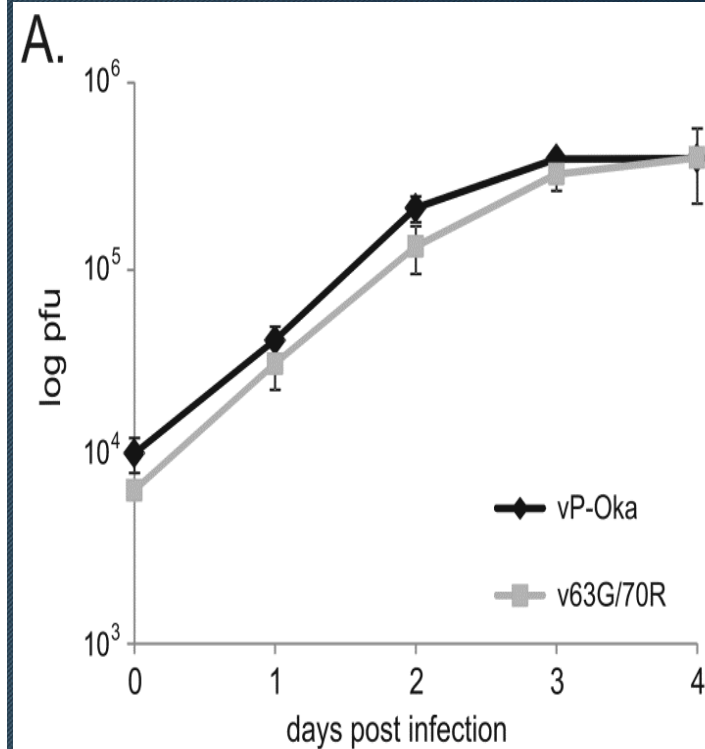
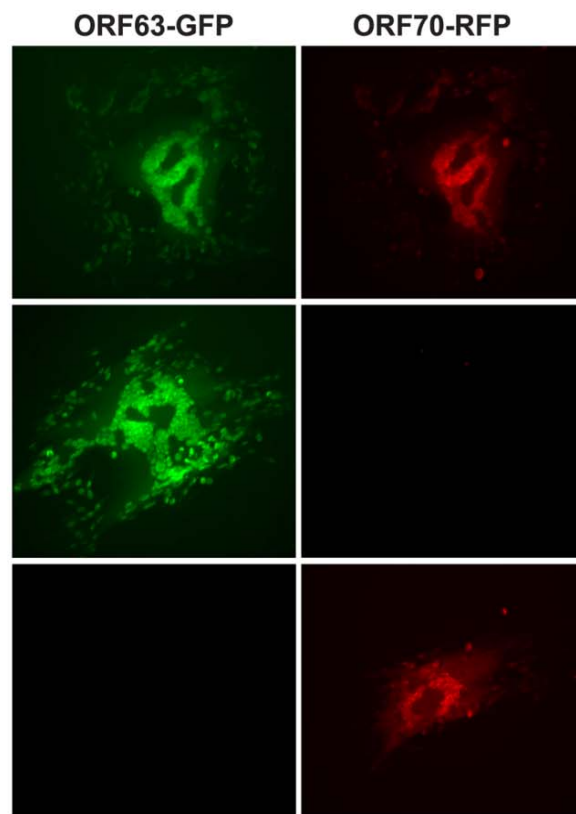
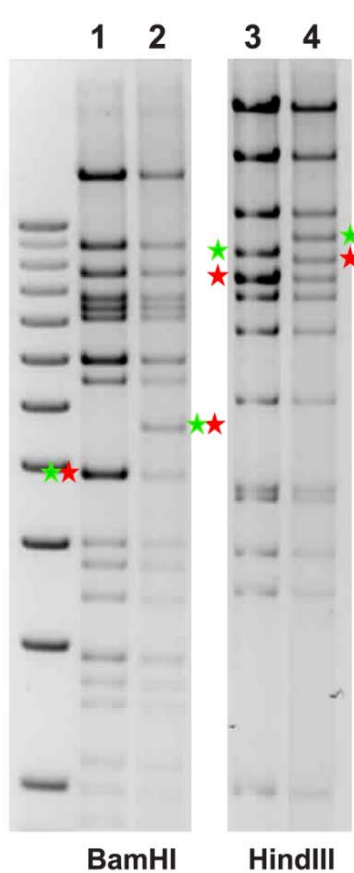
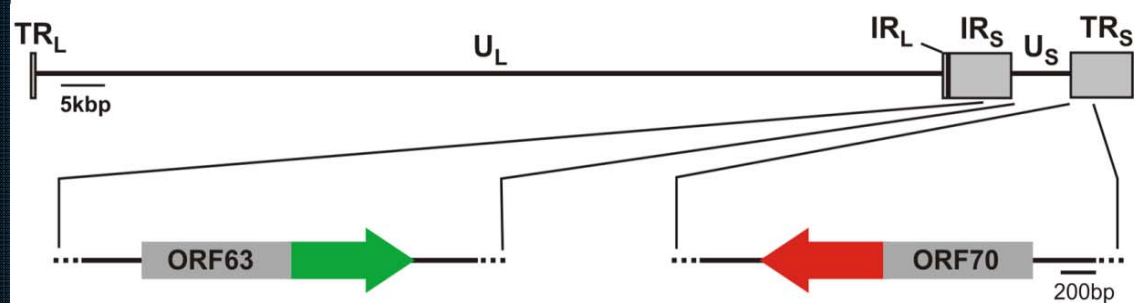
ORF63

ORF70



relative expression



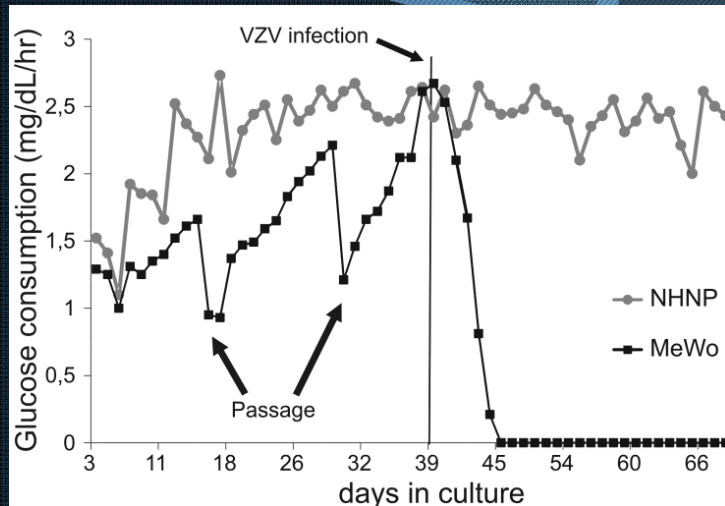


Goodwin TJ, McCarthy M, Osterrieder N, Cohrs RJ, Kaufer BB (2013), "Three-Dimensional Normal Human Neural Progenitor Tissue-Like Assemblies: A Model of Persistent Varicella-Zoster Virus Infection". *PLoS Pathogens* 9(8): e1003512. doi:10.1371/journal.ppat.1003512

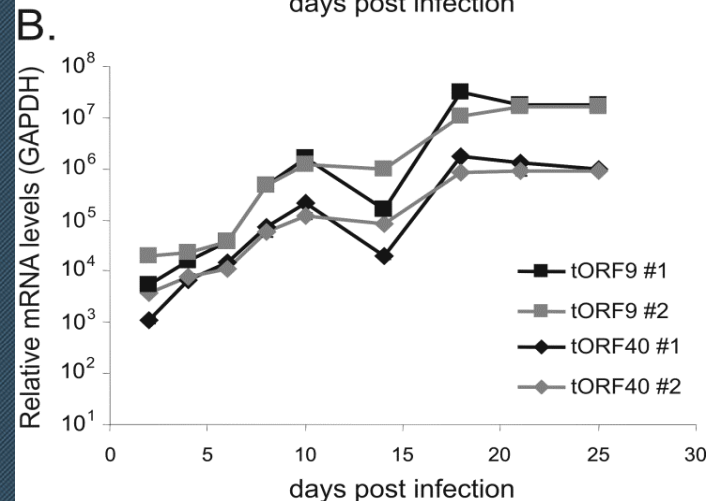
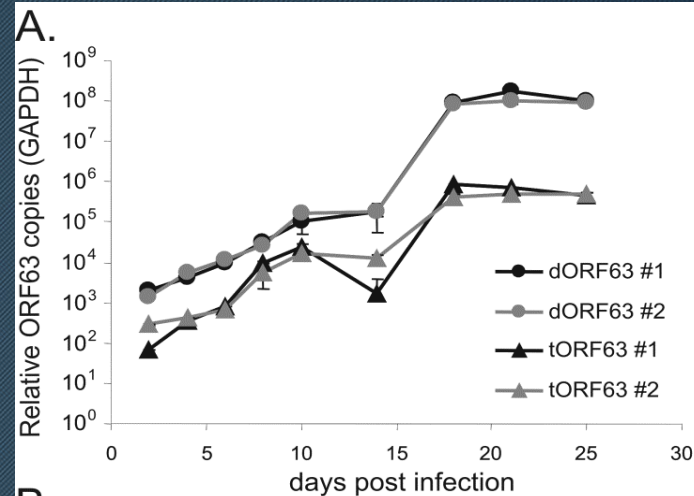




# 3D Normal Human Neural Progenitor Tissue-Like Assemblies: A Model of Persistent VZV Infection and a Platform to Study Oxidative Stress and Damage in Multiple Hit Scenarios



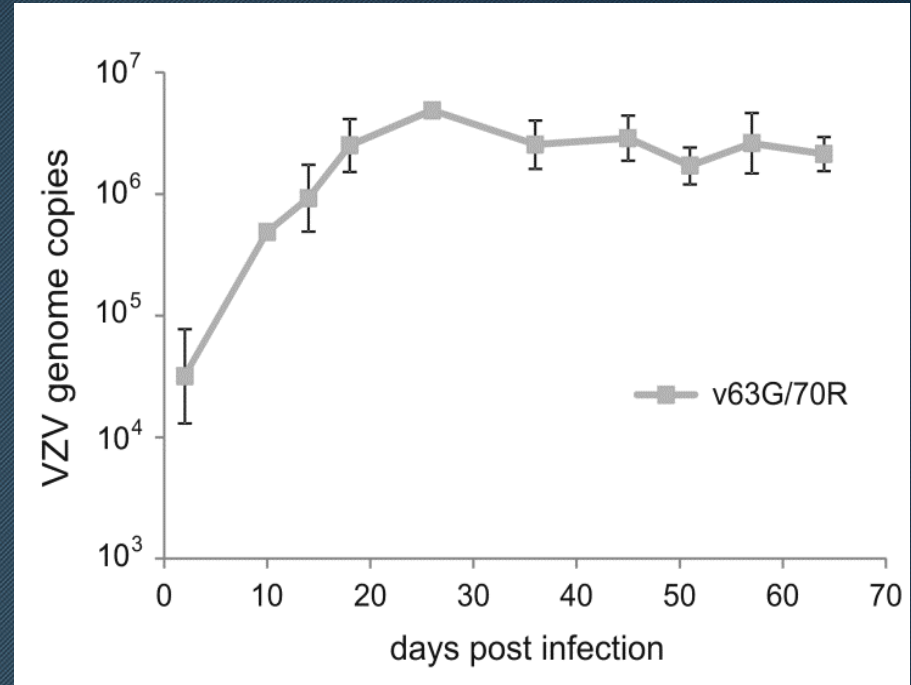
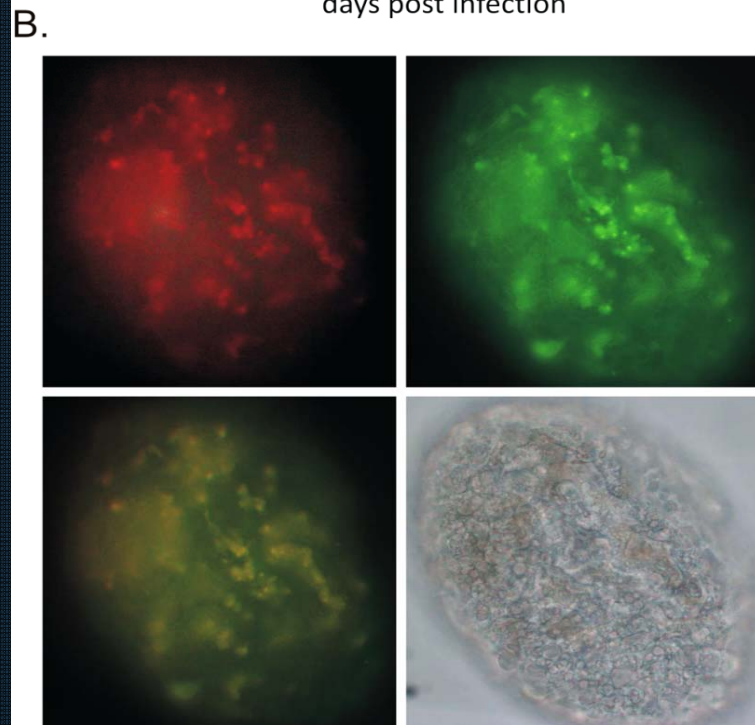
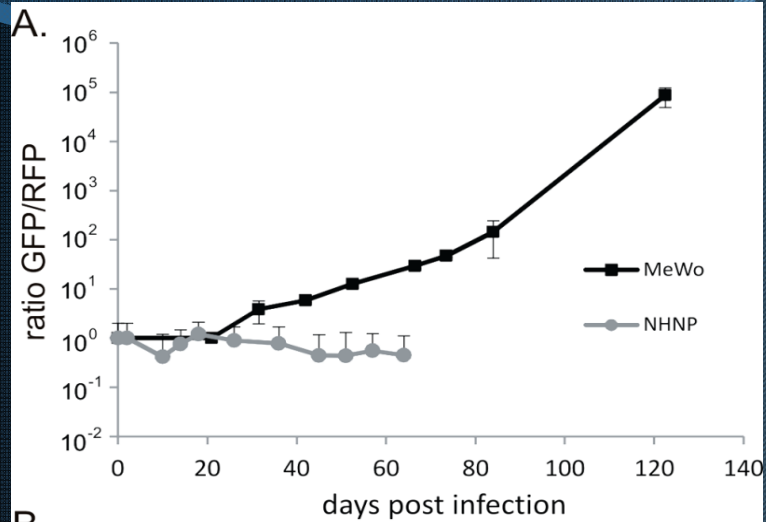
days post infection	plaque forming units per ml (5 wells)				
	1	2	3	4	5
0	371	351	207	402	457
2	5	2	2	1	2
4	3	0	0	2	2
6	1	2	2	2	1
10	0	1	0	0	2
14	0	0	0	0	0
21	0	0	0	0	0
25	2	1	1	3	2
30	0	0	0	0	0
35	0	0	1	1	0



cell free virus

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- Rearrangement recombination is frequent in MeWo cell
- Rearrangement recombination is absent in NHNP-TLA
- Both ORF63 and ORF70 are expressed in NHNP-TLA
- VZV-infected NHNP-TLA are stable for months

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## Conclusions

- The creation of a 3D neural model in the NASA bioreactor emulates the free fall conditions of microgravity (Application #1)
- The 3D NHNP progenitor model mimics aspects of the human Trigeminal Ganglia (TG) allowing a persistent extremely low level infection similar to VZV clinical latency (Application #2)
- 3D Tissue models of the near peripheral nervous system (nPNS, TG/NHNP) may serve as a viable platform to study VZV and other latent viruses re-expressed in the space environment. **Relevant for Immune Risks** (Application #3)
- Provides a nervous system model that is closely related to the CNS (by way of the trigeminal and optic nerves, Relevant to VIIP) and therefore is relevant to studying **CNS damage and Degen Risks from OSaD** in these tissues (Application #4)
- Provides a platform to study multiple hit scenarios of OSaD simultaneously (viral infection, radiation insult, and microgravity) on tissues relevant to HRP Risk and Gaps (Nutrition) and develop countermeasures (Application #5).





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